

## Prevalence of *Campylobacter* within a Swine Slaughter and Processing Facility<sup>†‡</sup>

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### ABSTRACT

In this work, the occurrence of *Campylobacter* in a swine slaughter and processing facility was studied. Thirty composite carcass samples, representing 360 swine carcasses, were taken immediately after exsanguination, immediately after polishing, after the final wash, and after overnight chilling at 2°C. Thirty matching composite rectal samples were also taken immediately after exsanguination, and 60 nonmatching individual colon samples were collected from the same lot of swine during evisceration. Also, 72 environmental samples were collected from equipment used in the slaughter operation (42 samples) and the processing operation (30 samples). *Campylobacter* was isolated by direct plating on Campy-Line agar (CLA) or Campy-Cefex agar (CCA), as well as by Bolton broth enrichment and subsequent inoculation onto CLA or CCA. For all four recovery methods combined, *Campylobacter* was detected on 33% (10 of 30) of the composite carcasses immediately after exsanguination, 0% (0 of 30) after polishing, 7% (2 of 30) immediately before chilling, and 0% (0 of 30) after overnight chilling. The pathogen was recovered from 100% (30 of 30) of the composite rectal samples and 80% (48 of 60) of the individual colon samples. *Campylobacter* was detected in 4.8% (2 of 42) and 3.3% (1 of 30) of the slaughter and processing equipment samples, respectively. The recovery rate achieved with direct plating on CLA was significantly higher ( $P < 0.05$ ) than those achieved with the other three recovery methods. For the 202 isolates recovered from all of the various samples tested, *Campylobacter coli* was the predominant species (75%) and was followed by *Campylobacter* spp. (24%) and *Campylobacter jejuni* (1%). These results indicate that although *Campylobacter* is highly prevalent in the intestinal tracts of swine arriving at the slaughter facility, this microorganism does not progress through the slaughtering operation and is not detectable on carcasses after overnight chilling.

*Campylobacter* is the leading cause of bacterial foodborne illness in the United States, resulting in an estimated 2,000,000 cases per year (19). Pathogenic species that cause human enteritis include *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari*, the so-called thermotolerant campylobacters (13), with *C. jejuni* accounting for 90 to 95% of all campylobacteriosis cases. *Campylobacter* is widely distributed in the environment and is frequently found in the intestinal tracts of animals (8, 15, 34). In fact, *Campylobacter* spp., primarily *C. coli*, are present in larger numbers than either *Salmonella* or *Yersinia* spp. in the intestinal tracts of swine (3).

Swine carcasses are commonly contaminated with feces at the slaughter facility (35), and feces can remain associated with the carcass as it progresses through the slaughter process. In addition, the potential exists for fecal material to leak from the intestines during the evisceration process. Thus, the pathogen may be transported on contam-

inated carcasses from the slaughtering operation to the food-processing operation and ultimately onto the final product. Carcass breaking and fabrication can also provide opportunities for fecal contamination of the final product (10, 12). Although it is well established that poultry are a vehicle for foodborne campylobacteriosis, the involvement of swine in foodborne campylobacteriosis is not well known. However, the incidence of *Campylobacter* on pork products at the retail level is estimated to be 1.3% (7). Therefore, pork may also be an important vehicle for human foodborne infection.

Little research on the incidence and distribution of *Campylobacter* in swine has been conducted, and to our knowledge the effects of carcass processing on the prevalence of *Campylobacter* at multiple sampling points in a swine slaughter facility have not yet been thoroughly investigated. It is also true that *Campylobacter* isolation procedures have yet to be standardized, and therefore numerous methods have been employed to isolate the organism. Current methods involve selective enrichment and selective plating (14) (both of which involve the use of antibiotics to suppress the growth of competing organisms) followed by biochemical and immunological confirmation. A selective and differential agar, Campy-Line agar (CLA), was developed for the isolation of *Campylobacter* from poultry

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carcass samples (17). Selective antibiotics are incorporated into CLA along with low levels of triphenyltetrazolium chloride (200 mg/liter); the former suppresses the growth of non-*Campylobacter* flora, and the latter allows the differentiation of *Campylobacter* from background flora.

The present study was designed to determine the prevalence and distribution of *Campylobacter* associated with swine at multiple points during slaughter and fabrication at a cooperating facility. Carcass, rectal, and fecal samples from the colons of corresponding swine, as well as environmental samples, were cultured as potential reservoirs of the organism. In addition, direct plating with CLA and CCA and enrichment methods involving a combination of Bolton broth and CLA or CCA were evaluated for their ability to recover and isolate this organism from swine samples.

## MATERIALS AND METHODS

**Sampling plan.** This study was conducted at a cooperating facility that slaughters and processes about 7,000 swine per day. The study was conducted over a 30-day period during the summer of 2001. Three to four lots of pigs were sampled on each of three visits, for a total of 10 lots and 360 carcasses. The general slaughtering techniques used in the facility included electrical stunning, exsanguination, scalding, dehairing, polishing, singeing, evisceration, washing, and overnight chilling. Processing techniques included deboning and fabrication. On each of three visits, carcass, rectal, colon, and environmental samples were collected. On day 1 of each visit, samples were collected from 120 carcasses (i) immediately after exsanguination (postkill), (ii) immediately after polishing (postpolish), and (iii) after the final wash (prechill) and were then composited in groups of 12 to yield 10 composite samples. Ten composite rectal swabs were collected from the same carcasses after the exsanguination step. In addition, 20 colons were collected from individual swine carcasses from the same lot during evisceration, and 14 environmental samples were collected from the dehairer, the polisher, the singer, and the final washer. On day 2 of each visit, samples were obtained from the same 120 carcasses and then composited in groups of 12 to yield 10 composite samples (iv) after overnight chilling (postchill). In addition, at about noon on each visit, 10 environmental samples were collected from conveyor belts and plastic cutting boards in the processing-fabrication room while the target lots were being further processed.

**Composite sponge sampling of carcass surfaces.** With minor modifications, carcass surfaces were sponge swabbed according to the U.S. Department of Agriculture–Food Safety and Inspection Service sponge protocol as described by Palumbo et al. (27). Briefly, 30-ml portions of sterile 0.1% peptone water (Difco Laboratories, Detroit, Mich.) were added to Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) containing three sterile sponges. Composite carcass samples were taken by surface swabbing a 100-cm<sup>2</sup> area on the outside of the neck on each of 12 carcasses with three sterile sponges; one sponge was used to swab each of four carcasses. Following sampling, the three sponges were returned to another sterile Whirl-Pak bag, which was placed on ice for transport to the laboratory. In total, 30 composite samples, representing 360 carcasses, were prepared.

**Composite fecal swab sampling.** Following electrical stunning of the animals, individual sterile swabs were used to collect feces from the rectum of each of the 12 corresponding surface-

swabbed carcasses. For each composite sample, 12 cotton swabs (Puritan, Hardwood Products Company, Maine) were moistened in 3 ml of sterile 0.1% sterile peptone water. A single cotton swab was used to sample each animal. After the samples had been collected, the 12 swabs were combined in the same Whirl-Pak bag and placed on ice for transport. In total, 30 composite samples, representing 360 carcasses, were prepared.

**Colon sampling.** Following evisceration, individual colon samples were obtained by removing a portion of the distal colon approximately 12.5 to 15 cm above the rectum with a sanitized knife. Each colon section was then placed into a sterile Whirl-Pak bag and placed on ice for transport. In total, 60 colons (20 for each of the three visits) were collected from the same lots of 360 carcasses from which the composite carcass and fecal samples were obtained.

**Sampling of slaughter and processing equipment.** Briefly, for the sampling of slaughter and processing equipment, 10-ml portions of sterile 0.1% peptone water were added to Whirl-Pak bags containing single sterile sponges. Environmental samples were obtained from slaughter equipment through the surface swabbing of 100-cm<sup>2</sup> areas of the dehairer and the polisher. In addition, 20-ml samples of water were collected aseptically from the dehairer, the singer, the polisher, and the final washer, and 10-g samples of detritus were collected from the dehairer. Environmental samples were also obtained from fabrication and processing equipment via the surface swabbing of 100-cm<sup>2</sup> areas of the conveyor belts and cutting boards. In total, 42 environmental samples were obtained from equipment used in the slaughter operation, and 30 environmental samples were obtained from the fabrication and processing equipment.

**Microbiological analyses.** The methods used to recover *Campylobacter* from each sample type were (i) direct plating on CLA, (ii) direct plating on CCA, (iii) Bolton broth enrichment and subsequent inoculation onto CLA, and (iv) Bolton broth enrichment and subsequent inoculation onto CCA. The CLA was prepared as described by Line (17), and the CCA was prepared as described by Stern et al. (33). Composite carcass, composite rectal, and environmental swab samples were massaged by hand for 1 min, and environmental water samples were vortexed. One gram of feces was aseptically removed from each colon with a sterile spatula and added to 4 ml of buffered peptone water (Oxoid, Basingstoke, UK). The entire contents of the bag were massaged by hand for 1 min to dislodge the feces from the spatula as well as to ensure even distribution of the sample in the buffered peptone water. Undiluted portions (100 µl each) of composite carcass samples, composite rectal samples, fecal samples from the colon, and environmental swab and water samples were plated directly onto both CLA and CCA. Plates were incubated at 42°C for 48 h under a microaerophilic atmosphere containing 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub> (CampyPak Plus, Becton Dickinson, Franklin Lakes, N.J.). The remaining portions of composite carcass and composite rectal samples, as well as environmental swab and water samples, were enriched in 100 ml of Bolton broth, while fecal samples from the colon were enriched in 10-ml volumes of Bolton broth. All samples were massaged by hand for 1 min and incubated aerobically for 4 h at 37°C and then for an additional 20 h at 42°C. After enrichment, an undiluted portion of each sample was directly plated onto both CLA and CCA with a sterile swab. Plates were incubated for 48 h at 42°C under a microaerophilic atmosphere. For each isolation method, typical colonies (up to 10 colonies per sample) were selected from each selective agar. *Campylobacter* colonies appear flat or slightly raised, shiny, and

deep magenta on CLA, whereas they appear grayish or light cream on CCA (33). For each suspected colony, wet mounts were prepared and examined. Presumptive isolation was indicated microscopically by the presence of spiral or S-shaped narrow (0.2- to 0.4- $\mu$ m) organisms exhibiting corkscrew-like motility. Biochemical tests included the catalase test (3% H<sub>2</sub>O<sub>2</sub>), the oxidase test (tetramethyl-*p*-phenylenediamine) (Difco), and the glucose fermentation test with triple sugar iron (Difco) slants. Colonies exhibiting the biochemical profile typical of *Campylobacter* (i.e., being positive for catalase and oxidase and negative for triple sugar iron), were further evaluated with latex agglutination (Indx-Campy(jcl), Integrated Diagnostics, Inc., Baltimore, Md.). Isolates showing positive agglutination were stored on cryoprotective beads (Key Scientific Products, UK) at -20°C.

**Confirmation of *Campylobacter* by polymerase chain reaction.** Presumptive *Campylobacter* isolates were grown on Mueller-Hinton agar (Difco), and the genomic DNA was extracted with the Prepman Sample Preparation Reagent (Perkin Elmer-Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Amplification reactions were performed with total volumes of 50  $\mu$ l with the use of the procedures and primers described by Cloak and Fratamico (6).

**Statistical analyses.** Data were analyzed to compare the four recovery methods with version 8.0 of the SAS statistical package (SAS Institute, Inc., Cary, N.C.). Comparisons of the percentages of positive results for the four methods for each sampling location and for all samples were carried out by the chi-square statistical method.

## RESULTS

A preliminary study was carried out to establish the optimal sampling site on a swine carcass for the recovery of *Campylobacter*. To this end, the recovery efficiency of individual and composite sponge samples collected from the ham, belly, and neck areas of carcasses following exsanguination and before and after chilling were compared. *Campylobacter* was not recovered when individual sponges were used; however, when composite sponges were applied to multiple carcasses, *Campylobacter* was more likely to be recovered from the neck area than from the belly or ham area (data not shown). On the basis of these results, the neck area of each carcass was sampled, and sponge samples from multiple carcasses were composited. The greater likelihood of recovering the pathogen from the neck area than from the ham or belly area may be due to the way swine carcasses are hung (i.e., head down) as they are moved through the slaughtering operation. With the carcass hung in this position, fecal material, water, and other debris deposited on carcasses will tend to collect in the neck area.

Through direct plating, *Campylobacter* was isolated from 13% (4 of 30) of the composite postkill carcass samples on CCA, compared with 33% (10 of 30 samples) on CLA (Table 1). Conversely, *Campylobacter* was not recovered from these same carcass samples after enrichment in Bolton broth followed by plating on either CLA or CCA. In corresponding rectal samples collected after exsanguination and then composited, the pathogen was isolated from 67% (20 of 30) and 63% (19 of 30) of the samples by direct plating on CCA and CLA, respectively. Following the enrichment of swabs from composited rectal samples in Bol-

TABLE 1. Prevalence of *Campylobacter* on swine-associated samples

Recovery method	Campylobacter prevalence for sample type <sup>a</sup>						
	Postkill <sup>b</sup>	Rectal <sup>c</sup>	Postpolish <sup>b</sup>	Prechill <sup>b</sup>	Postchill <sup>b</sup>	Colon <sup>d</sup>	Environmental slaughter <sup>e</sup>
Direct plating on CLA	33 (10/30)	63 (19/30)	0 (0/30)	3 (1/30)	0 (0/30)	58 (35/60)	0 (0/30)
Direct plating on CCA	13 (4/30)	67 (20/30)	0 (0/30)	0 (0/30)	0 (0/30)	37 (22/60)	0 (0/30)
Enrichment and plating on CLA	0 (0/30)	17 (5/30)	0 (0/30)	3 (1/30)	0 (0/30)	33 (20/60)	3 (1/30)
Enrichment and plating on CCA	0 (0/30)	47 (14/30)	0 (0/30)	0 (0/30)	0 (0/30)	43 (26/60)	3 (1/30)
Total <sup>g</sup>	33 (10/30)	100 (30/30)	0 (0/30)	7 (2/30)	0 (0/30)	80 (48/60)	3 (1/30)
							33 (93/282)

<sup>a</sup> Percentage of samples positive (number of samples positive/number of samples tested).

<sup>b</sup> Composite carcass samples ( $n = 30$ ).

<sup>c</sup> Composite rectal samples ( $n = 30$ ).

<sup>d</sup> Individual colon samples ( $n = 60$ ).

<sup>e</sup> Individual samples collected from slaughter equipment ( $n = 42$ ).

<sup>f</sup> Individual samples collected from fabrication equipment ( $n = 30$ ).

<sup>g</sup> The number of positive samples is not additive for a given column.

TABLE 2. Percentages of *Campylobacter* species isolated from positive swine samples regardless of recovery method

Sample type	No. of isolates/no. of positive samples <sup>a</sup>	% (no. of isolates of species/no. of isolates) of <i>Campylobacter</i> spp. <sup>b</sup>		
		<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter</i> spp.
Composite carcass (postkill)	15/10	73 (11/15)	7 (1/15)	20 (3/15)
Composite rectal	69/30	68 (47/69)	0 (0/69)	32 (22/69)
Composite carcass (prechill)	2/2	100 (2/2)	0 (0/20)	0 (0/2)
Colon samples	112/48	79 (89/112)	1 (1/112)	20 (22/112)
Environmental slaughter	2/2	50 (1/2)	0 (0/2)	50 (1/2)
Environmental fabrication	2/1	100 (2/2)	0 (0/2)	0 (0/2)
Total	202/93	75 (151/202)	1 (2/202)	24 (48/202)

<sup>a</sup> A positive sample is any sample from which *Campylobacter* was recovered, including some samples that tested positive by more than one method.

<sup>b</sup> *Campylobacter* isolates were speciated by both biochemical and PCR-based methods (6).

ton broth, *Campylobacter* was isolated from 47% (14 of 30) of the samples on CCA and from 17% (5 of 30) of the samples on CLA. The pathogen was not detected by any of the four recovery methods on composite postpolish carcass samples. *Campylobacter* was isolated from 3% (1 of 30) of the composite prechill carcass samples by direct plating on CLA and from 3% (1 of 30) of the carcass samples following enrichment in Bolton broth with subsequent plating on CLA. However, the pathogen was not detected on carcasses by direct plating on CCA or by enrichment with subsequent plating on CCA. *Campylobacter* was not detected on carcasses after overnight chilling regardless of the recovery method. A total of 60 individual colon samples were obtained after evisceration from the same lots of 360 carcasses. By the direct plating method, *Campylobacter* was isolated from 37% (22 of 60) of the colon samples on CCA and from 58% (35 of 60) of the colon samples on CLA. After enrichment in Bolton broth, the recovery rates were 43% (26 of 60 samples) for CCA and 33% (20 of 60 samples) for CLA.

*Campylobacter* was also recovered from equipment at two locations in the slaughter operation. Specifically, the pathogen was recovered from a water sample obtained from the polisher by direct plating on CCA and from the dehairer by direct plating on CLA; these two samples were collected on separate visits. In the fabrication-processing room, *Campylobacter* was recovered from a single cutting board on both CLA and CCA following enrichment in Bolton broth but not by direct plating on these same agar.

When the results of all four recovery methods were taken into consideration, the prevalence of *Campylobacter* on composite postkill carcass samples was 33% (10 of 30 samples), whereas the prevalence of the bacterium on composite prechill carcass samples was 7% (2 of 30 samples) (Table 1). The prevalence rates for the pathogen in composite rectal samples and individual colon samples were 100% (30 of 30 samples) and 80% (48 of 60 samples), respectively. The pathogen was recovered from 5% (2 of 42) of the environmental samples taken from the slaughter equipment and from 3% (1 of 30) of the environmental samples taken from the fabrication and processing equipment used in the facility. Overall, the prevalence of the

pathogen recovered from all of the swine-associated samples obtained in this study was 33% (93 of 282 samples).

*Campylobacter* was recovered from a significantly larger number ( $P < 0.05$ ) of swine-associated samples (23%; 66 of 282 samples) by direct plating on CLA than by the other three recovery methods. Also, direct plating on CCA plates (17%; 47 of 282 samples) and enrichment with subsequent direct plating on CCA plates (15%; 41 of 282 total samples) were significantly more efficient ( $P < 0.05$ ) in recovering the pathogen from samples than was enrichment with subsequent direct plating on CLA plates (10%; 27 of 282 samples).

A total of 202 isolates were obtained from the 93 samples that tested positive for *Campylobacter* by at least one of the four recovery methods. On the basis of identification with species-specific DNA primers and PCR, *C. coli* accounted for 73% (11 of 15) of the isolates recovered from the 10 positive composite postkill carcass samples, whereas *Campylobacter* spp. and *C. jejuni* accounted for 20% (3 of 15) and 7% (1 of 15) of these isolates, respectively (Table 2). In addition, *C. coli* was the only species recovered from the two positive composite prechill carcasses. *C. coli* was also the dominant species isolated from the 30 positive composite rectal samples (68%; 47 of 69 isolates), while *Campylobacter* spp. made up the remainder of these isolates (32%; 22 of 69 isolates). This same trend was also observed for the 48 positive colon samples: *C. coli* (79%; 89 of 112 isolates) > *Campylobacter* spp. (20%; 22 of 112 isolates) > *C. jejuni* (1%; 1 of 112 isolates). For the two positive environmental samples collected from slaughter equipment, *C. coli* was isolated from detritus collected from the dehairer, and *Campylobacter* spp. were isolated from water used in the polisher. Finally, for the environmental samples collected from equipment used in fabrication and processing, both isolates recovered from a single cutting board were *C. coli* isolates. Of the 202 isolates recovered in this study, 75% (151 of 202) were identified as *C. coli*, 24% (48 of 202) were identified as *Campylobacter* spp., and 1% (2 of 202) were identified as *C. jejuni*.

## DISCUSSION

In this study, four recovery methods were evaluated for their effectiveness in isolating *Campylobacter* from a

swine slaughter and processing facility. The results of all four methods combined indicated that 80% (48 of 60) of the colon samples and 100% (30 of 30) of the composite rectal samples contained *Campylobacter*. These findings are in agreement with results reported by Weijtens et al. (35), who, on the basis of ileal and rectal samples collected at the slaughterhouse, found that 85% of swine from eight Dutch farms were carriers of *Campylobacter*. In Norway, Rosef et al. (31) sampled feces from swine immediately after slaughter and recovered *Campylobacter* from 100% of the samples. In addition, Mafu et al. (18) found the organism in 99% of 200 fecal samples obtained from swine at a Canadian slaughterhouse. In the present study, the results of all four recovery methods indicated that the prevalence of the pathogen on the carcass surface after exsanguination was 33% (10 of 30 composite carcass samples). This value is within the range of prevalence levels obtained by Morgan and Krautil (20), who isolated *Campylobacter* from 33 to 76% of carcasses sampled after exsanguination in eight slaughterhouses. However, in the present study, *Campylobacter* levels were reduced to below detectable levels on the carcass surface after polishing. This reduction was most likely the result of the heat applied during the scalding and singeing steps, which directly precede the polishing step. Several studies have established that both of these thermal processes generate sufficient heat to significantly reduce bacterial levels (2, 29). Oosterom et al. (26) found that levels of *C. jejuni* on poultry skin pieces in a poultry processing plant were reduced by ca.  $3 \log_{10}$  CFU/cm<sup>2</sup> after the scalding process, and in the same plant, *Campylobacter* was not detected in scald tank water held at 58°C. Likewise, Sorquist and Danielsson-Tham (32) examined the survival of *Campylobacter* in scald tank water held at 56°C and reported a reduction of  $6 \log_{10}$  CFU/cm<sup>2</sup> after 1 to 1.25 min. In the present study, *Campylobacter* was also isolated from water in the polishing machine and from detritus that had accumulated on the frame of the dehairing machine. Gill and Bryant (11) examined dehairing equipment used in two slaughtering plants and found that detritus in the equipment contained 1 to  $6 \log_{10}$  CFU of *Campylobacter* per g, while the circulating waters contained up to  $2 \log_{10}$  CFU of the bacterium per ml. Morgan et al. (21) reported that fecal material can escape from the anus during the dehairing process. Therefore, fecal material, some of which may contain high levels of *Campylobacter*, may potentially reside within the laths and frames of the dehairing machine or in the circulating water.

*Campylobacter* was isolated from 7% of the composite prechill carcass samples by all four recovery methods combined. A possible explanation for the reappearance of the pathogen on carcasses after the final wash may be that feces containing the pathogen spread onto the carcass from the intestines during the removal of the intestinal tract (3). Although the scald tank, the dehairer, and the singer sufficiently eliminated the pathogen with the final polishing, the removal of the intestines during evisceration may have resulted in the spilling of fecal material and the recontamination of some of the carcasses. Following overnight chilling at 2°C, the pathogen was not detected on any composite

carcass samples. The reduction in *Campylobacter* prevalence as a result of chilling is consistent with the findings of Oosterom et al. (24) and Bracewell et al. (4), who found *Campylobacter* spp. on 9 and 13% of swine carcasses, respectively, before chilling and on 0% of the carcasses after chilling. One possible explanation for the reduction in the recovery rate for this particular pathogen after chilling may be the organism's sensitivity to drying as the air temperature is decreased (3, 28). For example, Oosterom et al. (25) reported that a 25 to 35% decrease in relative humidity resulted in a  $\sim 2\text{-log}_{10}$  decrease in *Campylobacter* levels on pig skin pieces cooled to 4°C within 24 h. For the 30 environmental samples collected from the processing-fabrication room in the present study, *C. coli* was recovered from a single cutting board. *C. coli* may have entered the processing-fabrication room on the shoes or clothing of processing-fabrication room employees who traveled through common-use areas such as bathrooms, hallways, and cafeterias that are also frequented by employees from the slaughtering area. Although unlikely, it is possible that the pathogen may have survived on a carcass during the overnight chilling. Regardless, within the scope of this study, *Campylobacter* was isolated at such an infrequent rate from the processing-fabrication room samples that it seems that carcass breaking-fabrication does not represent a major risk (9) of cross-contamination with *Campylobacter* in this particular facility.

In the present study, *C. coli* was isolated much more frequently (75%; 151 of 202 isolates) from the 93 positive samples than was *C. jejuni* (1%; 2 of 202 isolates). The remaining isolates were identified as *Campylobacter* spp. (24%; 48 of 202 isolates). These findings are similar to those of Cloak and Fratamico (6), who found *C. coli* in 87% of 60 isolates obtained from this same pork slaughter and processing facility, albeit 3 years earlier. Furthermore, *C. jejuni* was not isolated from rectal samples and was only present in 1% of colon samples. This finding is in agreement with the results of a study conducted by Ono and Yamamoto (23), who detected *C. coli* in 82.5% and *C. jejuni* in only 4.4% of the fecal contents of swine. Although swine may be only a minor reservoir of *C. jejuni*, most cases of human gastroenteritis are caused by this bacterium (5, 16). These data also suggest that *C. coli* is particularly adapted to the swine enteric environment.

The results of the present study demonstrate the ability and potential of *Campylobacter* to gain entry into a swine processing facility via the carcass surface or in fecal contents via the rectum and colon, leading to possible contamination of processing lines, equipment, and the finished product. However, these results also demonstrate that slaughtering techniques such as dehairing, singeing, and chilling are effective in the reduction or elimination of the pathogen as the carcass progresses through the slaughtering operation. *Campylobacter* recovery rates reported in the present study cannot be considered absolute, since several factors, including seasonality, age, sample type (i.e., composite carcass and rectal versus individual colon, solid versus liquid, and sponge versus swab), feeding regime, geographical variation, and especially recovery method and

medium, may appreciably affect the isolation of the organism. Procedures for the recovery of *Campylobacter* have yet to be standardized, and a variety of isolation and detection media are currently in use. In the present study, direct plating on CLA recovered the pathogen from a significantly larger ( $P < 0.05$ ) number of samples than did direct plating on CCA or enrichment in Bolton broth with subsequent plating on CCA or CLA. A possible explanation for the difference between the two agars used in the direct plating method with respect to recovery rates is the incorporation of triphenyltetrazolium chloride, which some *Campylobacter*, as well as many other microorganisms, are able to reduce from a colorless salt to the insoluble compound formazon, in CLA plates (17). The production of formazon imparts a red color to *Campylobacter* colonies, allowing easier identification and selection of *Campylobacter* from among background flora than is possible with CCA plates. To our knowledge, this is the first study to evaluate the performance of CLA in the recovery of *Campylobacter* from swine samples. In a study involving poultry carcass rinse samples conducted by CLA's inventor (17), no significant difference ( $P < 0.05$ ) between CLA and CCA was detected. In addition, direct plating on CLA may have an advantage over methods involving an enrichment step, because intestinal microflora may be present in larger numbers and/or may be more vigorous and outgrow *Campylobacter* during incubation in the enrichment media, particularly under aerobic conditions such as those used here. This may make it more difficult to recover the target organism on the selective medium after enrichment, thereby confounding detection. This observation is supported by the fact that most enrichment broths are not fully selective and can support the growth of other organisms (1). Musgrove et al. (22), in a study of poultry cecal contents, also found that enrichment yielded significantly fewer *Campylobacter*-positive samples (63% of samples positive) than did direct plating (100% of samples positive).

Although direct plating on CLA was significantly ( $P < 0.05$ ) more effective in recovering *Campylobacter* than were the other three methods examined, the results of the present study also indicate that the use of multiple recovery methods can increase the rate of recovery of *Campylobacter* from swine samples. For example, the highest recovery rate for colon samples for a single method was 58%, whereas a combination of all four methods increased the recovery rate for colon samples to 80%. Similarly, the highest recovery rate for rectal samples for one method was 67%, whereas *Campylobacter* was recovered from 100% of the samples when a combination of methods was used. It is also clear from the results of the present investigation that the prevalence of the organism will vary depending on both the method and the medium used for its isolation or detection. It is therefore possible that the prevalence of *Campylobacter* on swine is frequently underreported. A potential shortcoming of the use of a combination of methods is that such an approach is very costly, time-consuming, and laborious and therefore may not be feasible when a large number of samples are involved. Regardless, our data confirm that *Campylobacter* is highly prevalent on the surfaces

and in the intestinal tracts of swine arriving at the slaughter facility. However, for an initial carcass prevalence of 33%, slaughter procedures were sufficient to eliminate *Campylobacter* from carcass surfaces, and *Campylobacter* was not detected after chilling, indicating that chilling is an efficient critical control point for this organism. Studies to optimize the methods and media used to recover *Campylobacter* from swine and to chronicle the source, persistence, and clonality of these microorganisms are ongoing.

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